## REMARKS

Applicants respectfully request reconsideration of the present application.

### 1. Disposition of the Claims and Specification

Claims 21-30 and 35-37 are currently pending. Claims 1-20 and 31 are canceled. Claims 32-34 and 38-42 are withdrawn from consideration. Claims 21 and 30 are amended. Support for the amendment to claim 21 may be found in the specification, for example, at page 1, line 9, page 21, lines 9-14, page 22, lines 23-26, and in Table 2. Support for the amendment to claim 30 may be found in the specification, for example, at page 1, line 9, page 20, lines 26-31, and at page 22, lines 30-33.

Because the foregoing amendments do not introduce new matter, entry thereof by the Examiner is respectfully requested.

### 2. Claim Rejections – 35 U.S.C. § 101

Claims 21-31 and 35-37 remain rejected under 35 U.S.C. § 101 because the claimed invention is allegedly not supported by either a specific and substantial asserted utility or a well established utility, for reasons of record as stated in the previous Office Action (Paper 15, pages 5-8). In the previous Office Action, the examiner asserted that the specification discloses the claimed protein as a "cell surface receptor, based on the presence of a putative secretory signal sequence, transmembrane domain and homology to a known receptor, non-CD4 glycoprotein gp120 receptor. However, there is no activity attributed to the protein, no teaching of any specific disease or disorder correlated with the protein and no disclosure of any protein or molecule (such as a ligand) that interacts with it. Therefore, the nucleic acid, encoded protein and methods of use do not have any specific and substantial utility, or a well established utility." See Paper No. 15 at page 5. Applicants respectfully disagree with the examiner and request reconsideration of the rejection.

**a. SEQ ID NO: 2 is homologous to CD209L**

According to the results of the attached sequence alignment, performed with SEQ ID NO: 12 of the instant invention, the protein identified in the specification having an amino acid sequence corresponding to SEQ ID NO: 12 is 81% identical to CD209L. *See* Alignment (Exhibit 1). The annotation of CD209L (Exhibit 2) shows that CD209L is a receptor for ICAM3 and a virus attachment and presentation factor, as well as plays a role in the infective process and pathogenicity of viruses such as HIV and Ebola.

The CD209L annotation also provides several synonyms for CD209L, including DC-SIGNR and L-SIGN. Bashirova *et al.*, A Dendritic Cell-Specific Intercellular Adhesion Molecule 3-grabbing Nonintegrin (DC-SIGN)-related Protein is Highly Expressed on Human Liver Sinusoidal Endothelial Cells and Promotes HIV-1 Infection, J. EXPERIMENTAL MEDICINE, 193(6): pp. 671-678 (2001), (attached hereto as Exhibit 3 and previously submitted in an IDS on 10/21/2002), discloses DC-SIGNR and L-SIGN as close homologues with the same function. Bashirova *et al.* at pages 675-677.

**b. Alvarez *et al.* disclose that DC-SIGN/gp120 facilitates HIV and Ebola infection**

Alvarez *et al.*, C-Type Lectins DC-SIGN and L-SIGN Mediate Cellular Entry by Ebola Virus in *cis* and in *trans*, J. VIROLOGY, pp. 6841-44 (2002), explains that DC-SIGN (dendritic cell [DC]-specific ICAM-3 grabbing non-integrin, CD209) is a type II membrane protein with a C-type lectin extracellular domain. Alvarez *et al.* also explain that DC-SIGN was "originally cloned as a human immunodeficiency virus (HIV) gp120-binding protein", and it "facilitate[s] infection in *trans* of susceptible cells." Alvarez *et al.* at page 6841. Studies with cells expressing DC-SIGN, and a related homologue, L-SIGN, show that these proteins also facilitate infection of the Ebola virus. Alvarez *et al.* at page 6842-43.

Similarly, page 31, lines 18-20 and page 32, lines 7-10 of the specification discloses that the claimed polypeptide and polynucleotides may be administered to treat a disorder associated with increased HCSRP activity such as "an infection caused by a viral agent classified as a filovirus ... [or] retrovirus." Further, Table 2 of the specification specifically

links the claimed polypeptide of SEQ ID NO: 12 to the gp120 receptor, which according to Alvarez *et al.*, is the same as DC-SIGN. Therefore, Alvarez *et al.* confirm that the polypeptide of SEQ ID NO: 12 does have a specific and substantial utility in the treatment of a disease caused by HIV or the Ebola virus.

**c. Pohlmann *et al.* disclose that DC-SIGNR, even though only 77% similar to DC-SIGN, also facilitates HIV infection**

Pohlmann *et al.*, DC-SIGNR, a DC-SIGN Homologue Expressed in Endothelial Cells, Binds to Human and Simian Immunodeficiency Viruses and Activates Infection in Trans, PNAS, 98(5): pp. 2670-2675 (2001), explains that "a homologue of DC-SIGN, termed DC-SIGNR (for DC-SIGN related), that exhibits 77% amino acid identity with DC-SIGNR ... also functions as a universal attachment factor for primate lentiviruses that can bind and transmit multiple HIV-1, HIV-2, and SIV strains to receptor-positive cell lines." Pohlmann *et al.* at page 2670, 2673.

Similarly, page 31, lines 18-20 and page 32, lines 7-10 of the specification discloses that the claimed polypeptide and polynucleotides may be administered to treat a disorder associated with increased HCSRP activity such as "an infection caused by a viral agent classified as a filovirus ... [or] retrovirus." Further, Table 2 of the specification specifically links the claimed polypeptide of SEQ ID NO: 12 to the gp120 receptor. Because Pohlmann *et al.* teach that DC-SIGN and DC-SIGNR have the same function, even though only 77% identical, SEQ ID NO: 12, which is 81% identical to DC-SIGNR, should also have the same function as that associated with DC-SIGN and DC-SIGNR. Therefore, Pohlmann *et al.* confirm that the polypeptide of SEQ ID NO: 12 has a specific and substantial utility of treating HIV and Ebola infections.

**d. Bashirova *et al.* teaches that DC-SIGN/gp120 and DC-SIGNR/L-SIGN have the same function and bind the same HIV ligand**

Bashirova *et al.*, confirms that DC-SIGN is "identical to the previously reported type II membrane-associated C-type lectin that binds HIV-1 envelope glycoprotein gp120 in a CD4-independent manner." Bashirova *et al.* at page 671. Further, the authors believe that a

more appropriate name for the DC-SIGNR molecule is L-SIGN (liver/lymph node-specific ICAM-3-grabbing nonintegrin), based on the determination that the protein is expressed in liver and lymph node tissues. Bashirova *et al.* at page 672. The authors hypothesized and proved that L-SIGN would have the same function and bind similar ligands as that of DC-SIGN, even though only 77% similar, based on "the nearly identical amino acid sequence of their extracellular domains." Bashirova *et al.* at pages 675-677.

Similarly, page 31, lines 18-20 and page 32, lines 7-10 of the specification discloses that the claimed polypeptide and polynucleotides may be administered to treat a disorder associated with increased HCSRP activity such as "an infection caused by a viral agent classified as a filovirus ... [or] retrovirus." Further, Table 2 of the specification specifically links the claimed polypeptide of SEQ ID NO: 12 to the gp120 receptor.

**e. Alvarez *et al.*, Pohlmann *et al.*, and Bashirova *et al.* confirm the specific and substantial utility for SEQ ID NO: 12 as asserted in the specification**

The present specification describes the claimed polypeptide as belonging to the class of human cell surface receptors, and more specifically, those that "recognize antigens" and "bind ligands to be internalized by the cell." See page 1, lines 7-18. The specification also discloses the claimed polypeptide, represented by SEQ ID NO: 12, as a "Non-CD4 glycoprotein gp120 receptor." See Table 2. As described above, the claimed polypeptide is 81% identical to CD209L, which itself is the same as, or related to, DC-SIGN/DC-SIGNR/L-SIGN/gp120 receptor.

Page 31, lines 18-20 and page 32, lines 7-10 of the specification discloses that the claimed polypeptide and polynucleotides may be administered to treat a disorder associated with increased HCSRP activity such as "an infection caused by a viral agent classified as a ... filovirus ... [or] retrovirus."

As shown by Bashirova *et al.*, Pohlmann *et al.*, and Alvarez *et al.*, each of the proteins described above (DC-SIGN and its homologue DC-SIGNR) have a well-known function in the art, with respect to viral pathogenesis and infectivity. Specifically, these proteins are

implicated in the cellular binding of retroviruses such as HIV, and in the binding of filoviruses, such as Ebola. Further, as shown by Bashirova *et al.* and Pohlmann *et al.*, DC-SIGN has the same viral binding and recognition function as DC-SIGNR, even though only 77% identical to the DC-SIGNR protein. Similarly, the claimed polypeptide of SEQ ID NO: 12, which is even more identical to DC-SIGNR (81% identical), should also have the same viral binding and recognition function as DC-SIGNR. As such, one of skill in the art would understand that these proteins, including the protein represented by SEQ ID NO: 12, can be used in the prevention and treatment of diseases caused by viruses such as the HIV and/or Ebola virus.

Therefore, Applicants assert that the protein does have a specific, substantial, and well-established utility, namely for the treatment and/or prevention of diseases caused by the HIV virus and/or the Ebola virus. Reconsideration and withdrawal of the rejection is respectfully requested.

3. **Claim Rejections – 35 U.S.C. § 112, first paragraph**

The examiner has also rejected claims 21-31 and 35-37 under 35 U.S.C. § 112, first paragraph, because the claimed invention is not supported by either a specific and substantial asserted utility or a well-established utility. The examiner also reasons that one of skill in the art would not know how to use nucleic acids and polypeptides that are 85% identical to SEQ ID NO: 12 and SEQ ID NO: 25, or fragments of SEQ ID NO: 12.

Applicants have shown that the claimed polynucleotides and polypeptides have a well-established utility, as described above. Similarly, Applicants assert that the fragments and nucleic acids and polypeptides that are 95% identical to SEQ ID NO: 12 and 25 (as currently claimed), also have the same well-established utility, of the treatment of diseases caused by HIV and/or Ebola virus infection. Because the fragments, nucleic acids and polypeptides of SEQ ID NOs 12 and 25 are associated with the Ebola virus and HIV, a person of skill in the art would find these fragments, nucleic acids and polypeptides useful in the treatment of HIV and Ebola infections. The § 112, first paragraph rejection is therefore improper and should be withdrawn.

**4. Claim Rejections – 35 U.S.C. § 112, first paragraph**

The examiner has rejected claims 21, 23, 26, 27, 28, 30, 35 and 37 under 35 U.S.C. § 112, first paragraph for reasons of record in the previous Office Action. Previously, the examiner explained that the specification adequately describes the polynucleotide and polypeptide of SEQ ID NOs 12 and 25. However, the examiner did not believe that the disclosure of a single polypeptide adequately supported the scope of the claimed genus, which included various fragments and homologues of SEQ ID NO: 12.

The examiner states that a "description of a genus of cDNAs may be achieved by means of a representative number of cDNAs, defined by nucleotide sequence, falling within the scope of the genus, *or* of a recitation of structural features common to the genus, which features constitute a substantial portion of the genus." Office Action at 20. The examiner also explains that the recited structure of SEQ ID NO: 12 "combined with a functional limitation would usually provide adequate written description. However, in the instant case, having homology to the gp120 receptor does not mean that the instant claimed protein binds gp120, and such was not disclosed in the specification." Office Action at 20-21.

Applicants respectfully disagree with the examiner. However, to expedite prosecution, Applicants have amended claims 21 and 30 to recite variants that are 95% identical to SEQ ID NO: 12 and 25, rather than 85% identical. Further, Applicants have amended claims 21 and 30 to recite that the polypeptide binds an extracellular ligand. Support for these claim amendments may be found in the specification, for example, at page 1, line 9, page 20, lines 26-31, page 21, lines 9-14, and page 22, lines 23-26 and lines 30-33.

With respect to the examiner's assertion that a genus may be adequately described by reciting "structural features common to the genus," Applicants point to Table 2 of the instant specification. Table 2 recites "structural features common to the genus" such as: (1) potential phosphorylation sites (S56, T185, T234, S289, S76, S211, T245, S248); (2) potential glycosylation sites (N64, N287); and (3) various motifs, signature sequences and protein domains such as a transmembrane domain (Q26-V44), a signal peptide domain (M1-S52); and a 2 poly-immunoglobulin receptor (M1-P48; A22-K69; V95-G142; V118-G165).

In summary, Applicants assert that the specification does "provide sufficient distinguishing identifying characteristics of the genus" such as "disclosure of complete or partial structure, physical and/or chemical properties," and "functional characteristics." Office Action at 21. Accordingly, Applicants respectfully request reconsideration and withdrawal of the rejection.

## 5. Claim Rejections – 35 U.S.C. § 112, second paragraph

The examiner has rejected claims 21-30 and 35-37 under 35 U.S.C. § 112, second paragraph for reasons of record in a previous office action. Specifically, the examiner believes that it is not clear what is meant by the term "naturally occurring" in claims 21 and 30. Applicants respectfully disagree with the examiner. However, to expedite prosecution, Applicants have amended claims 21 and 30. The present version of the claims avoids this issue. The rejection should be withdrawn.

## 6. Conclusion

Applicants believe that the present application is in condition for allowance. Favorable reconsideration of the application as amended is respectfully requested. The Examiner is invited to contact the undersigned by telephone if it is felt that a telephone interview would advance the prosecution of the present application.

The Commissioner is hereby authorized to charge any additional fees which may be required regarding this application under 37 C.F.R. §§ 1.16-1.17, or credit any overpayment, to Deposit Account No. 19-0741. Should no proper payment be enclosed herewith, as by a check being in the wrong amount, unsigned, post-dated, otherwise improper or informal or

even entirely missing, the Commissioner is authorized to charge the unpaid amount to Deposit Account No. 19-0741. If any extensions of time are needed for timely acceptance of papers submitted herewith, Applicants hereby petition for such extension under 37 C.F.R. §1.136 and authorizes payment of any such extensions fees to Deposit Account No. 19-0741.

Respectfully submitted,

Date 9/7/04

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**3344986CD1_PRT_12_PF-0636-USN** 325 aa

**CD209L** 399 aa

CD209 antigen-like, a receptor for ICAM3 and a virus attachment and presentation factor that may play a role in the infective process and pathogenicity of HIV, hepatitis C, and Ebola viruses

Match: Length=399, Identity: 81%, Similarity:81%, Query Overlap: 100%, Subject Overlap: 100%, E-value:0.0, Score:615

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Query: 1   MSDSKEPRVQQLGLL----------------------------GCLGHGALVLQLLSFML 32
           MSDSKEPRVQQLGLL                            GCLGHGALVLQLLSFML
Sbjct: 1   MSDSKEPRVQQLGLLEEDPTTSGIRLFPRDFQFQQIHGHKSSTGCLGHGALVLQLLSFML 60

Query: 33  LAGVLVAILVQVSKVPSSLSQEQSEQDAIYQNLTQLKAAVGELSEKSKLQEIYQELTQLK 92
           LAGVLVAILVQVSKVPSSLSQEQSEQDAIYQNLTQLKAAVGELSEKSKLQEIYQELTQLK
Sbjct: 61  LAGVLVAILVQVSKVPSSLSQEQSEQDAIYQNLTQLKAAVGELSEKSKLQEIYQELTQLK 120

Query: 93  AAVGELPEKSKL----------------------QEIYQELTRLKAAVGELPEKSKLQE 129
           AAVGELPEKSKL                      QEIYQELTRLKAAVGELPEKSKLQE
Sbjct: 121 AAVGELPEKSKLQEIYQELTRLKAAVGELPEKSKLQEIYQELTRLKAAVGELPEKSKLQE 180

Query: 130 IYQELTR----------------------LKAAVGELPEKSKLQEIYQELTRLKAAVGE 166
           IYQELTR                     LKAAVGELPEKSKLQEIYQELT+LKAAVGE
Sbjct: 181 IYQELTRLKAAVGELPEKSKLQEIYQELTELKAAVGELPEKSKLQEIYQELTQLKAAVGE 240

Query: 167 LPDQSKQQQIYQELTDLKTAFERLCRHCPKDWTFFQGNCYFMSNSQRNWHDSVTACQEVR 226
           LPDQSKQQQIYQELTDLKTAFERLCRHCPKDWTFFQGNCYFMSNSQRNWHDSVTACQEVR
Sbjct: 241 LPDQSKQQQIYQELTDLKTAFERLCRHCPKDWTFFQGNCYFMSNSQRNWHDSVTACQEVR 300

Query: 227 AQLVVIKTAEEQNFLQLQTSRSNRFSWMGLSDLNQEGTWQWVDGSPLSPSFQRYWNSGEP 286
           AQLVVIKTAEEQNFLQLQTSRSNRFSWMGLSDLNQEGTWQWVDGSPLSPSFQRYWNSGEP
Sbjct: 301 AQLVVIKTAEEQNFLQLQTSRSNRFSWMGLSDLNQEGTWQWVDGSPLSPSFQRYWNSGEP 360

Query: 287 NNSGNEDCAEFSGSGWNDNRCDVDNYWICKKPAACFRDE 325
           NNSGNEDCAEFSGSGWNDNRCDVDNYWICKKPAACFRDE
Sbjct: 361 NNSGNEDCAEFSGSGWNDNRCDVDNYWICKKPAACFRDE 399
```

Schematic Colors:

| Very Strong | Strong | High | Moderate | Low | Weak |
|---|---|---|---|---|---|
| >95% | 80-95% | 45-80% | 35-45% | 25-35% | 20-25% |

BKL Annotation details of one docket matching protein

**Human CD209L CD209 antigen-like, a receptor for ICAM3 and a virus attachment and presentation factor that may play a role in the infective process and pathogenicity of HIV, hepatitis C, and Ebola viruses**

**Disease**

**Molecular Mechanism:**

viral exploitation of the sugar binding of CD209L may cause increased viral spread within host, cell to cell associated with HIV Infections *2001* (11581425)

viral exploitation of the sugar binding of CD209L may cause increased viral spread within host, cell to cell associated with ebola hemorrhagic fever *2002* (12050398)

viral exploitation of the sugar binding of CD209L may cause increased virion attachment associated with ebola hemorrhagic fever *2002* (12050398)

viral exploitation of the sugar binding of CD209L may cause Hepatitis C *2003* Pharm (12676990)

**Phenotype**

**Title line phrases**

**Membership:**

C-type lectin family *2001* (11226297)

**Molecular/Biochemical Function:**

binds to ICAM3 *2001* (11257134)

**Role in Disease:**

a potential antiretroviral target *2001* (11226297)

plays a role in the infective process and pathogenicity of Ebola virus *2002* (12050398)

binds to multiple strains of HIV 1, HIV 2 and simian immunodeficiency virus and transmits these viruses to both T cell lines and peripheral blood mononuclear cells *2001* (11226297)

**Synonyms**

HP10347
DC-SIGNR
LSIGN
DCSIGNR
DC-SIGN2
MGC47866

**Cognate members**

**GO**

GO ontology: mannose binding *Experimental (E) 2001* (11739956)
GO ontology: mannose binding *Sequence Similarity (S) 2000* (10975799)
GO ontology: receptor activity *Experimental (E) 2001* (11257134)
GO ontology: protein binding *Experimental (E) 2001* (11257134)
GO ontology: integral to plasma membrane *Experimental (E) 1999* (10072769)
GO ontology: response to virus *Experimental (E) 2001* (11257134)
GO ontology: antigen presentation *Predicted (P) 2001* (11226297)
alternative form: mRNA editing *Experimental (E) 2001* (11337487)

**Expression**

Cell origin: cell line * Degree: not * Techniques: rt-PCR * Cell types: T-lymphocytes *Experimental (E) 2001* (11337487)
Cell types: endothelium/endothelial cells * Techniques: immunolocalization * Body: placenta *Experimental (E) 2001* (11226297)
Cell origin: cell line * Tumors: monocytic leukemia * Techniques: rt-PCR *Experimental (E) 2001* (11337487)
Techniques: cDNA isolation * Body: placenta *Experimental (E) 2001* (11337487)
Tumors: adenocarcinoma * Cell origin: cell line * Degree: not * Techniques: rt-PCR *Experimental (E) 2001* (11337487)
Degree: high * Techniques: protein detected * Misc. Organ/Cell Type: liver sinusoidal cells *Experimental (E) 2001* (11257134)
Body: liver * Cell types: endothelium/endothelial cells *Experimental (E) 2001* (11257134)
Misc. Organ/Cell Type: liver sinusoidal endothelial cells *Experimental (E) 2001* (11257134)
Body: lymph node * Cell types: endothelium/endothelial cells * Techniques: immunolocalization *Experimental (E) 2001* (11226297)
Body: liver * Cell types: endothelium/endothelial cells * Techniques: immunolocalization *Experimental (E) 2001* (11226297)
Body: brain * Cell types: endothelium/endothelial cells * Techniques: mRNA detected * Cell origin: primary cells in culture *Experimental (E) 2002* (12083838)
Cell types: B-lymphocytes * Cell origin: cell line * Degree: not * Techniques: rt-PCR *Experimental (E) 2001* (11337487)
Cell types: leukocytes * Degree: not * Techniques: rt-PCR *Experimental (E) 2001* (11337487)
Body: blood * Cell types: dendritic cells * Techniques: rt-PCR *Experimental (E) 2001* (11337487)
Body: liver * Techniques: mRNA detected *Experimental (E) 2001* (11257134)
Body: lymph node * Techniques: mRNA detected *Experimental (E) 2001* (11257134)
Degree: not * Cell types: dendritic cells * Techniques: mRNA detected *Experimental (E) 2001* (11257134)
Degree: high * Techniques: mRNA detected * Misc. Organ/Cell Type: liver sinusoidal cells *Experimental (E) 2001* (11257134)
Body: liver * Techniques: protein detected *Experimental (E) 2001* (11257134)
Body: lymph node * Techniques: protein detected *Experimental (E) 2001* (11257134)
Degree: not * Cell types: dendritic cells * Techniques: protein detected *Experimental (E) 2001* (11257134)
Body: placenta * Techniques: immunolocalization *Experimental (E) 2001* (11226297)
Techniques: immunolocalization * Misc. Organ/Cell Type: liver sinusoidal endothelial cells * Misc. Organ/Cell Type: endothelial cells of lymph node sinuses * Misc. Organ/Cell Type: placentalvilli *Experimental (E) 2001* (11226297)

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**References**

**Primary isolated human brain microvascular endothelial cells express diverse HIV/SIV-associated chemokine coreceptors and DC-SIGN and L-SIGN.**
MUKHTAR, M; HARLEY, S; CHEN, PQ; BOUHAMDAN, M; PATEL, C;

ACHEAMPONG, E; POMERANTZ, RJ // Thomas Jefferson Univ & Univ Penn & Wayne State Univ: Virology 2002 May 25;297(1):78-88. (12083838)

**DC-SIGNR, a DC-SIGN homologue expressed in endothelial cells, binds to human and simian immunodeficiency viruses and activates infection in trans.** POHLMANN, S; SOILLEUX, EJ; BARIBAUD, F; LESLIE, GJ; MORRIS, LS; TROWSDALE, J; LEE, B; COLEMAN, N; DOMS, RW // Univ Cambridge & Univ Penn: Proc Natl Acad Sci U S A 2001 Feb 27;98(5):2670-2675. (11226297)

**Structural basis for selective recognition of oligosaccharides by DC-SIGN and DC-SIGNR.** FEINBERG, H; MITCHELL, DA; DRICKAMER, K; WEIS, WI // Stanford Univ & Univ Oxford: Science 2001 Dec 7;294(5549):2163-6. (11739956)

Pharm **L-SIGN (CD 209L) is a liver-specific capture receptor for hepatitis C virus.** GARDNER, JP; DURSO, RJ; ARRIGALE, RR; DONOVAN, GP; MADDON, PJ; DRAGIC, T; OLSON, WC // Progen Pharmaceut Inc & Yeshiva Univ Albert Einstein Coll Med: Proc Natl Acad Sci U S A 2003 Apr 15;100(8):4498-503. Epub 2003 Apr 3. (12676990)

**Selection of cDNAs encoding putative type II membrane proteins on the cell surface from a human full-length cDNA bank.** YOKOYAMA-KOBAYASHI, M; YAMAGUCHI, T; SEKINE, S; KATO, S // ERATO & Sagami Chem Res Ctr: Gene 1999 Mar 4;228(1-2):161-7 (10072769)

**DC-SIGN; a related gene, DC-SIGNR; and CD23 form a cluster on 19p13.** SOILLEUX, EJ; BARTEN, R; TROWSDALE, J // Univ Cambridge: J Immunol 2000 Sep 15;165(6):2937-42 (10975799)

**A dendritic cell-specific intercellular adhesion molecule 3-grabbing nonintegrin (dc-sign)-related protein is highly expressed on human liver sinusoidal endothelial cells and promotes hiv-1 infection.** BASHIROVA, AA; GEIJTENBEEK, TBH; VAN DUIJNHOVEN, GCF; VAN VLIET, SJ; EILERING, JBG; MARTIN, MP; WU, L; MARTIN, TD; VIEBIG, N; KNOLLE, PA; KEWALRAMANI, VN; VAN KOOYK, Y; CARRINGTON, M // NCI & Univ Nijmegen & Zentrum Mol Biol Heidelberg: J Exp Med 2001 Mar 19;193(6):671-8. (11257134)

**C-type lectins DC-SIGN and L-SIGN mediate cellular entry by Ebola virus in cis and in trans.** ALVAREZ, CP; LASALA, F; CARRILLO, J; MUNIZ, O; CORBI, AL; DELGADO, R // CSIC & Hosp 12 Octubre: J Virol 2002 Jul;76(13):6841-4. (12050398)

**Extensive repertoire of membrane-bound and soluble dendritic cell-specific ICAM-3-grabbing nonintegrin 1 (DC-SIGN1) and DC-SIGN2 isoforms. Inter-individual variation in expression of DC-SIGN transcripts.** MUMMIDI, S; CATANO, G; LAM, L; HOEFLE, A; TELLES, V; BEGUM, K; JIMENEZ, F; AHUJA, SS; AHUJA, SK // S Texas Vet Hlth Care Syst & Univ Texas: J Biol Chem 2001 Aug 31;276(35):33196-212. (11337487)

**DC-SIGN interactions with human immunodeficiency virus: virus binding and transfer are dissociable functions.** POHLMANN, S; LESLIE, GJ; EDWARDS, TG; MACFARLAN, T; REEVES, JD; HIEBENTHAL-MILLOW, K; KIRCHHOFF, F; BARIBAUD, F; DOMS, RW // Univ Erlangen Nurnberg & Univ Penn: J Virol 2001 Nov;75(21):10523-6. (11581425)

**Uncurated References**

**A novel mechanism of carbohydrate recognition by the C-type lectins DC-SIGN and DC-SIGNR - Subunit organization and binding to multivalent ligands** MITCHELL, DA; FADDEN, AJ; DRICKAMER, K // Univ Oxford: 2001; JOURNAL OF BIOLOGICAL CHEMISTRY 276(31) p.28939-28945 (ISI link)

**Functional and antigenic characterization of human, rhesus macaque, pigtailed macaque, and murine DC-SIGN** BARIBAUD, FD; POHLMANN, S; SPARWASSER, T; KIMATA, MTY; CHOI, YK; HAGGARTY, BS; AHMAD, N; MACFARLAN, A; EDWARDS, TG; LESLIE, GJ; ARNASON, J; REINHART, TA; KIMATA, JT; LITTMAN, DR; HOXIE, JA; DOMS, RW // NYU Med Ctr & SW Fdn Biomed Res & Univ Penn & Univ Pittsburgh: 2001; JOURNAL OF VIROLOGY 75 (21) p.10281-10289 (ISI link)

**Five mouse homologues of the human dendritic cell C-type lectin, DC-SIGN** PARK, CG; TAKAHARA, K; UMEMOTO, E; YASHIMA, Y; MATSUBARA, K; MATSUDA, Y; CLAUSEN, BE; INABA, K; STEINMAN, RM // Hokkaido Univ & Kyoto Univ & Rockefeller Univ: 2001; INTERNATIONAL IMMUNOLOGY 13(10) p.1283-1290 (ISI link)

**Expression of DC-SIGN by dendritic cells of intestinal and genital mucosae in humans and rhesus macaques** JAMESON, B; BARIBAUD, F; POHLMANN, S; GHAVIMI, D; MORTARI, F; DOMS, RW; IWASAKI, A // R&D Syst & Univ Penn & Yale Univ: 2002; JOURNAL OF VIROLOGY 76(4) p.1866-1875 (ISI link)

Pharm **Functional evaluation of DC-SIGN monoclonal antibodies reveals DC-SIGN interactions with ICAM-3 do not promote human immunodeficiency virus type 1 transmission** WU, L; MARTIN, TD; VAZEUX, R; UNUTMAZ, D; KEWALRAMANI, VN // ICOS Corp & NCI & Vanderbilt Univ: 2002; JOURNAL OF VIROLOGY 76(12) p.5905-5914 (ISI link)

**The mannose-dependent epitope for neutralizing antibody 2G12 on human immunodeficiency virus type 1 glycoprotein gp120** SANDERS, RW; VENTURI, M; SCHIFFNER, L; KALYANARAMAN, R; KATINGER, H; LLOYD, KO; KWONG, PD; MOORE, JP // Cornell Univ & Mem Sloan Kettering Canc Ctr & NIAID & Univ Agr & Forestry & Univ Amsterdam: 2002; JOURNAL OF VIROLOGY 76(14) p.7293-7305 (ISI link)

**Expression of human immunodeficiency virus (HIV)-binding lectin DC-SIGNR: Consequences for HIV infection and immunity** SOILLEUX, EJ; MORRIS, LS; RUSHBROOK, S; LEE, B; COLEMAN, N // Addenbrookes Hosp & Fred Hutchinson Canc Res Ctr & UCLA: 2002; HUMAN PATHOLOGY 33(6) p.652-659 (ISI link)

Pharm **Molecular characterization of the murine SIGNR1 gene encoding a C-type**

**lectin homologous to human DC-SIGN and DC-SIGNR** PARENT, SA; ZHANG, T; CHREBET, G; CLEMAS, JA; FIGUEROA, DJ; KY, B; BLEVINS, RA; AUSTIN, CP; ROSEN, H // Merck Res Labs: 2002; GENE 293(1-2) p.33-46 (ISI link)

**Quantitative expression and virus transmission analysis of DC-SIGN on monocyte-derived dendritic cells** BARIBAUD, F; POHLMANN, S; LESLIE, G; MORTARI, F; DOMS, RW // R&D Syst & Univ Penn: 2002; JOURNAL OF VIROLOGY 76(18) p.9135-9142 (ISI link)

**Marginal zone macrophages express a murine homologue of DC-SIGN that captures blood-borne antigens in vivo** GEIJTENBEEK, TBH; GROOT, PC; NOLTE, MA; VAN VLIET, SJ; GANGARAM-PANDAY, ST; VAN DUIJNHOVEN, GCF; KRAAL, G; VAN OOSTERHOUT, AJM; VAN KOOYK, Y // Univ Med Ctr St Radboud & Univ Utrecht & Vrije Univ Med Ctr Amsterdam: 2002; BLOOD 100(8) p.2908-2916 (ISI link)

**Human cytomegalovirus binding to DC-SIGN is required for dendritic cell infection and target cell trans-infection** HALARY, F; AMARA, A; LORTAT-JACOB, H; MESSERLE, M; DELAUNAY, T; HOULES, C; FIESCHI, F; ARENZANA-SEISDEDOS, F; MOREAU, JF; DECHANET-MERVILLE, J // INRA & Inst Pasteur & Univ Bordeaux 2 & Univ Grenoble 1 & Univ Halle Wittenberg: 2002; IMMUNITY 17(5) p.653-664 (ISI link)

**Novel member of the CD209 (DC-SIGN) gene family in primates** BASHIROVA, AA; WU, L; CHENG, J; MARTIN, TD; MARTIN, MP; BENVENISTE, RE; LIFSON, JD; KEWALRAMANI, VN; HUGHES, A; CARRINGTON, M // NCI & Univ S Carolina: 2003; JOURNAL OF VIROLOGY 77(1) p.217-227 (ISI link)

**Differential N-linked glycosylation of human immunodeficiency virus and Ebola virus envelope glycoproteins modulates interactions with DC-SIGN and DC-SIGNR** LIN, G; SIMMONS, G; POHLMANN, S; BARIBAUD, F; NI, HP; LESLIE, GJ; HAGGARTY, B; BATES, P; WEISSMAN, D; HOXIE, JA; DOMS, RW // Univ Penn: 2003; JOURNAL OF VIROLOGY 77(2) p.1337-1346 (ISI link)

**DC-SIGN and DC-SIGNR bind Ebola glycoproteins and enhance infection of macrophages and endothelial cells** SIMMONS, G; REEVES, JD; GROGAN, CC; VANDENBERGHE, LH; BARIBAUD, F; WHITBECK, JC; BURKE, E; BUCHMEIER, MJ; SOILLEUX, EJ; RILEY, JL; DOMS, RW; BATES, P; POHLMANN, S // Scripps Res Inst & USA & Univ Cambridge & Univ Penn: 2003; VIROLOGY 305(1) p.115-123 (ISI link)

**Transplacental transmission of HIV: a potential role for HIV binding lectins** SOILLEUX, EJ; COLEMAN, N // MRC: 2003; INTERNATIONAL JOURNAL OF BIOCHEMISTRY & CELL BIOLOGY 35(3) p.283-287 (ISI link)

**Hepatitis C virus glycoproteins interact with DC-SIGN and DC-SIGNR** POHLMANN, S; ZHANG, J; BARIBAUD, F; CHEN, ZW; LESLIE, G; LIN, G; GRANELLI-PIPERNO, A; DOM, RW; RICE, CM; MCSKEATING, JA // Aaron Diamond AIDS Res Ctr & Rockefeller Univ & Univ Penn: 2003; JOURNAL OF

VIROLOGY 77(7) p.4070-4080 (ISI link)

**DC-SIGN (CD209) mediates dengue virus infection of human dendritic cells** TASSANEETRITHEP, B; BURGESS, TH; GRANELLI-PIPERNO, A; TRUMPFHERER, C; FINKE, J; SUN, W; ELLER, MA; PATTANAPANYASAT, K; SARASOMBATH, S; BIRX, DL; STEINMAN, RM; SCHLESINGER, S; MAROVICH, MA // Henry M Jackson Fdn Adv Mil Med Dis & Mahidol Univ & Naval Med Res Ctr & Rockefeller Univ & Walter Reed Army Inst Res: 2003; JOURNAL OF EXPERIMENTAL MEDICINE 197(7) p.823-829 (ISI link)

**DC-SIGN and L-SIGN are high affinity binding receptors for hepatitis C virus glycoprotein E2** LOZACH, PY; LORTAT-JACOBS, H; DE LAVALETTE, AD; STAROPOLI, I; FOUNG, S; AMARA, A; HOULES, C; FIESCHI, F; SCHWARTZ, O; VIRELIZIER, JL; ARENZANA-SEISDEDOS, F; ALTMEYER, R // Inst Pasteur & Stanford Univ & UJF: 2003; JOURNAL OF BIOLOGICAL CHEMISTRY 278(22) p.20358-20366 (ISI link)

**Isolation and characterization of the human DC-SIGN and DC-SIGNR promoters** LIU, HB; YU, WD; LIOU, LY; RICE, AP // Baylor Coll Med: 2003; GENE 313() p.149-159 (ISI link)

**DC-SIGN and L-SIGN can act as attachment receptors for alphaviruses and distinguish between mosquito cell- and mammalian cell-derived viruses** KLIMSTRA, WB; NANGLE, EM; SMITH, MS; YUROCHKO, AD; RYMAN, KD // Louisiana State Univ: 2003; JOURNAL OF VIROLOGY 77(22) p.12022-12032 (ISI link)

**HCV and HIV binding lectin, DC-SIGNR, is expressed at all stages of HCV induced liver disease** COLE, G; COLEMAN, N; SOILLEUX, E // Hutchison MRC Res Ctr: 2004; JOURNAL OF CLINICAL PATHOLOGY 57(1) p.79-80 (ISI link)

**Human macrophage C-type lectin specific for galactose and N-acetylgalactosamine promotes filovirus entry** TAKADA, A; FUJIOKA, K; TSUIJI, M; MORIKAWA, A; HIGASHI, N; EBIHARA, H; KOBASA, D; FELDMANN, H; IRIMURA, T; KAWAOKA, Y // Canadian Sci Ctr Human & Anim Hlth & Univ Tokyo & Univ Wisconsin: 2004; JOURNAL OF VIROLOGY 78(6) p.2943-2947 (ISI link)

**Dynamic populations of dendritic cell-specific ICAM-3 grabbing nonintegrin-positive immature dendritic cells and liver/lymph node-specific ICAM-3 grabbing nonintegrin-positive endothelial cells in the outer zones of the paracortex of human lymph nodes** ENGERING, A; VAN VLIET, SJ; HEBEDA, K; JACKSON, DG; PREVO, R; SINGH, SK; GEIJTENBEEK, TBH; VAN KRIEKEN, H; VAN KOOYK, Y // Free Univ Amsterdam & John Radcliffe Hosp & Univ Nijmegen: 2004; AMERICAN JOURNAL OF PATHOLOGY 164(5) p.1587-1595 (ISI link)

**Characterization of a novel C-type lectin-like gene, LSECtin - Demonstration of carbohydrate node** LIU, WL; TANG, L; ZHANG, G; WEI, HD; CUI, YF; GUO, LH; GOU, ZK; CHEN, XX; JIANG, DF; ZHU, YP; KANG, GF; HE, FC // Beijing

Inst Radiat Med & Chongqing Univ Med Sci & Sun Yat Sen Univ & Tsing Hua Univ: 2004; JOURNAL OF BIOLOGICAL CHEMISTRY 279(18) p.18748-18758 (ISI link)

**Hepatitis C virus targets DC-SIGN and L-SIGN to escape lysosornal degradation**
LUDWIG, IS; LEKKERKERKER, AN; DEPLA, E; BOSMAN, F; MUSTERS, RJP; DEPRAETERE, S; VAN KOOYK, Y; GEIJTENBEEK, TBH // Free Univ Amsterdam & Innogenet NV: 2004; JOURNAL OF VIROLOGY 78(15) p.8322-8332 (ISI link)

# A Dendritic Cell–specific Intercellular Adhesion Molecule 3–grabbing Nonintegrin (DC-SIGN)–related Protein Is Highly Expressed on Human Liver Sinusoidal Endothelial Cells and Promotes HIV-1 Infection

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## Abstract

The discovery of dendritic cell (DC)-specific intercellular adhesion molecule (ICAM)-3–grabbing nonintegrin (DC-SIGN) as a DC-specific ICAM-3 binding receptor that enhances HIV-1 infection of T cells in trans has indicated a potentially important role for adhesion molecules in AIDS pathogenesis. A related molecule called DC-SIGNR exhibits 77% amino acid sequence identity with DC-SIGN. The *DC-SIGN* and *DC-SIGNR* genes map within a 30-kb region on chromosome 19p13.2-3. Their strong homology and close physical location indicate a recent duplication of the original gene. Messenger RNA and protein expression patterns demonstrate that the DC-SIGN–related molecule is highly expressed on liver sinusoidal cells and in the lymph node but not on DCs, in contrast to DC-SIGN. Therefore, we suggest that a more appropriate name for the DC-SIGN–related molecule is L-SIGN, liver/lymph node–specific ICAM-3–grabbing nonintegrin. We show that in the liver, L-SIGN is expressed by sinusoidal endothelial cells. Functional studies indicate that L-SIGN behaves similarly to DC-SIGN in that it has a high affinity for ICAM-3, captures HIV-1 through gp120 binding, and enhances HIV-1 infection of T cells in trans. We propose that L-SIGN may play an important role in the interaction between liver sinusoidal endothelium and trafficking lymphocytes, as well as function in the pathogenesis of HIV-1.

Key words: L-SIGN • adhesion receptor • chromosome 19p13.2-3 • ICAM-3 • HIV-1 gp120

## Introduction

Dendritic cell (DC)[1]-specific intercellular adhesion molecule (ICAM)-3–grabbing nonintegrin (DC-SIGN) has recently been identified as a DC-specific adhesion receptor that mediates the interaction between DCs and resting T cells through high affinity binding to ICAM-3, thereby facilitating the initiation of primary immune responses (1, 2). DC-SIGN was shown to be identical to the previously reported type II membrane-associated C-type lectin (2) that binds HIV-1 envelope glycoprotein gp120 in a CD4-independent manner (3). The affinity of DC-SIGN exceeds that of CD4 for HIV-1 gp120 (3), and upon capture of HIV-1, DC-SIGN does not appear to promote viral entry into the DC itself, but rather enhances infection of T cells in trans (1). DC-SIGN–associated HIV-1 remains infectious over a prolonged period of time, perhaps contributing to the infectious potential of the virus during its transport by DCs from the periphery to lymphoid organs.

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[1]*Abbreviations used in this paper:* CCR, CC chemokine receptor; DC, dendritic cell; DC-SIGN, DC-specific ICAM-3–grabbing nonintegrin; EST, expressed sequence tag; ICAM, intercellular adhesion molecule; L-SIGN, liver/lymph node–specific ICAM-3–grabbing nonintegrin; LSEC, liver sinusoidal endothelial cell; nt, nucleotides; RH, radiation hybrid; RT, reverse transcription; UTR, untranslated region.

A previous search by Yokoyama-Kobayashi et al. (4) for cDNA clones encoding type II membrane proteins resulted in the identification of a partial clone that was homologous, but not identical, to the cDNA encoding the molecule now known as DC-SIGN. The putative protein product contained a deletion of 28 amino acids in the cytoplasmic domain and was lacking the entire C-type lectin domain relative to the cDNA encoding DC-SIGN. More recently, Soilleux et al. (5) described the full-length cDNA sequence of the related gene, which they called *DC-SIGNR*. The genomic organization of *DC-SIGN* and *DC-SIGNR* was compared, indicating a high degree of similarity. Concomitant expression of the two genes in placenta, endometrium, and stimulated KG1 cells (a cell line that phenotypically resembles myeloid DCs) was observed, although the expression of *DC-SIGNR* was very low in both endometrium and stimulated KG1 cells (5).

While attempting to identify polymorphisms in the *DC-SIGN* gene, we also discovered the gene corresponding to the partial cDNA sequence described by Yokoyama-Kobayashi et al. (4). Tissue expression patterns of the *DC-SIGNR* gene indicated that it is expressed at considerably high levels in only two tissues, liver and lymph node, but not in monocyte-derived DCs. Therefore, we have called the molecule L-SIGN, liver/lymph node–specific ICAM-3–grabbing nonintegrin, which we believe more accurately depicts the function and expression pattern of this molecule than does DC-SIGNR. Here we refine the genomic organization of the *SIGN* gene complex, and also report the tissue distribution and functional characterization of the L-SIGN molecule.

## Materials and Methods

*Characterization of DC-SIGN and L-SIGN cDNA.* We have submitted the full *DC-SIGN* and *L-SIGN* cDNA sequences to GenBank/EMBL/DDBJ under accession nos. AF290886 and AF290887, respectively. The *L-SIGN* cDNA sequence represents a variant containing six repeats in exon 4. The 5′ and 3′ ends of the transcripts (except the 3′ end of the *DC-SIGN* mRNA) were determined by 5′ rapid amplification of cDNA ends (RACE; CLONTECH Laboratories, Inc.). The length of the 3′ end of the *DC-SIGN* mRNA was estimated based on Northern blot analysis data (transcript size) and reverse transcription (RT)-PCR data using forward primers specific for the 1.3-kb *DC-SIGN* cDNA sequence (3) and reverse primers specific for several GenBank/EMBL/DDBJ expressed sequence tags (ESTs) (e.g., AI472111 and AA454170), mapping downstream of the alleged 3′ end of *DC-SIGN*. A cDNA fragment containing the full coding sequence of *L-SIGN* (nucleotides [nt] 39–1184, GenBank/EMBL/DDBJ accession no. AF290887) was amplified from human placental mRNA (CLONTECH Laboratories, Inc.) and cloned into the expression vectors pcDNA3.1/V5-His/TOPO (pcDNA3-L-SIGN) and pCDM8 (pCDM8-L-SIGN).

*Radiation Hybrid Mapping.* PCR-based radiation hybrid (RH) mapping with *DC-SIGN*– and *L-SIGN*–specific primers was performed using the Genebridge 4 RH panel (Research Genetics). The PCR results were submitted to the Gene Map server at the Sanger Center (http://www.sanger.ac.uk/Software/Rhserver). The chromosomal position of markers linked to the genes was determined searching the Genatlas database (http://web.citi2.fr/GENATLAS) and the genetic map of human chromosome 19 provided by the Marshfield Clinic (http://research.marshfieldclinic.org/genetics/).

*Genotype Analysis of L-SIGN and DC-SIGN Exon4.* The repeat region in exon 4 was amplified with the following pairs of primers: L28, TGTCCAAGGTCCCCAGCTCCC, and L32, GAACTCACCAAATGCAGTCTTCAAATC, for *L-SIGN*; DL27, TGTCCAAGGTCCCCAGCTCC, and DI4R, CCCCGTGTTCTCATTTCACAG, for *DC-SIGN*. The cycle conditions were as follows: 94°C for 5 s and 68°C for 1 min. Alleles were distinguished by agarose gel electrophoresis and ethidium bromide staining.

*Northern Blot Analysis.* Total RNA from cultured human immature DCs (see below) was isolated using Trizol (Life Technologies). 10 μg of the isolated RNA was electrophoresed on a 1% agarose gel, transferred to Hybond-XL (Amersham Pharmacia Biotech) as described (6), and used for Northern blot analysis along with two human multiple tissue Northern blots (CLONTECH Laboratories, Inc.). Three probes were subsequently hybridized to the blots: (1) an *L-SIGN*–specific probe (nt 100–183, GenBank/EMBL/DDBJ accession no. AF290887); (2) a probe recognizing both *DC-SIGN* and *L-SIGN* (nt 1–1233, GenBank/EMBL/DDBJ accession no. AF290886); and (3) an actin control probe (CLONTECH Laboratories, Inc.). Hybridization procedures were performed according to manufacturer specifications (CLONTECH Laboratories, Inc.).

*Antibodies.* Anti–DC-SIGN mAbs AZN-D1 and AZN-D2 were described previously (2). mAb AZN-D3 was obtained by screening hybridoma supernatants of BALB/c mice immunized with THP-1–DC-SIGN cells (1) for the ability to stain both DC-SIGN and L-SIGN. Anti–DC-SIGN mAb AZN-D2 also cross-reacts with L-SIGN, as was initially determined by the staining of K562–L-SIGN cells (data not shown). Anti–L-SIGN rabbit antiserum was generated by immunization with two L-SIGN–specific peptides, PTTSGIRLFPRD and WNDNRCDVDNYW (Veritas, Inc. Laboratories).

*Cells.* DCs were cultured from monocytes in the presence of 500 U/ml IL-4 and 800 U/ml GM-CSF (Schering-Plough; references 7 and 8). At day 7 the cells expressed high levels of MHC class I and II, αMβ2 (CD11b), αXβ2 (CD11c), DC-SIGN and ICAM-1, moderate levels of LFA-1 and CD86, and low levels of CD14, as measured by flow cytometry. Stable K562 transfectants expressing L-SIGN (K562–L-SIGN) were generated by cotransfection of K562 with the pCDM8–L-SIGN plasmid and the pGK-neo vector by electroporation (9). Stable K562–DC-SIGN transfectants were generated in a similar manner using pRc/CMV–DC-SIGN (2). THP-1–DC-SIGN cells were described previously (2). Stable THP-1–L-SIGN transfectants were generated by electroporation of THP-1 cells with pcDNA3–L-SIGN, selection for G418 resistance, and positive sorting for L-SIGN expression using mAb AZN-D3. All cell lines were maintained in RPMI 1640 supplemented with 10% fetal bovine serum in addition to specific cytokine or antibiotic requirements as indicated. K562 and THP-1 are monocytic cell lines. HEK293T are human embryonic kidney cells containing a single temperature-sensitive allele of SV-40 large T antigen. GHOST cells are HIV-indicator cells derived from human osteosarcoma cells (10). Hut/CC chemokine receptor (CCR)5 cells are the transformed human T cell line Hut78 stably transduced with CCR5.

*Fluorescent Beads Adhesion Assay.* Carboxylate-modified TransFluorSpheres (488/645 nm, 1.0 μm; Molecular Probes) were coated with ICAM-3 as described previously for ICAM-1

(11). Fluorescent beads were coated with M-tropic HIV-$1_{MN}$ envelope glycoprotein gp120 as follows: streptavidin-coated fluorescent beads were incubated with biotinylated $F(ab')_2$ fragment rabbit anti–sheep IgG (6 μg/ml; Jackson ImmunoResearch Laboratories) followed by an overnight incubation with sheep anti-gp120 antibody D7324 (Alto Bio Reagents, Ltd.) at 4°C. The beads were washed and incubated with 250 ng/ml purified HIV-1 gp120 (provided by Immunodiagnostics, Inc., through the National Institutes of Health AIDS Research and Reference Reagent Program) overnight at 4°C. The fluorescent beads adhesion assay was performed as described by Geijtenbeek et al. (11). In brief, cells were resuspended in adhesion buffer (20 mM Tris-HCl, pH 8.0, 150 mM NaCl, 1 mM $CaCl_2$, 2 mM $MgCl_2$, 0.5% BSA) at a final concentration of $5 \times 10^6$ cells/ml. 50,000 cells were preincubated with mAb (20 μg/ml) for 10 min at room temperature. Ligand-coated fluorescent beads (20 beads/cell) were added and the suspension was incubated for 30 min at 37°C. Adhesion was determined by measuring the percentage of cells that bound fluorescent beads using flow cytometry on a FACScan™ (Becton Dickinson).

*Detection of L-SIGN on Primary Human Liver Sinusoidal Endothelial Cells.* Liver tissue was obtained from a patient undergoing liver surgery after having received written consent. Isolation of primary human liver cells was performed as described previously (12). Cells were cultured on collagen type I–coated tissue culture plates in supplemented Williams E Medium (13). The day after isolation, liver cells were incubated with Texas red–labeled OVA (10 μg/ml; Molecular Probes) for 2 h and detached from the matrix by gentle trypsin treatment. Cells were stained with rabbit anti–L-SIGN antiserum followed by goat anti–rabbit Ig FITC (Dianova) and analyzed with a FACScan™ (Becton Dickinson) using CELLQuest™ software. OVA uptake was characteristic of liver sinusoidal endothelial cells (LSECs) only and not Kupffer cells, as verified by the costaining of $OVA^+$ cells with an endothelial cell–specific marker, acetylated LDL, using confocal microscopy.

*HIV-1 Infection Assays.* The infection assays were performed as described previously (1, 2). Pseudotyped HIV-1 stocks were generated by calcium phosphate transfections of HEK293T cells with the proviral vector plasmid NL-Luc-$E^-R^-$ containing a firefly luciferase reporter gene (14) and expression plasmids for either ADA or JRFL gp160 envelopes. Viral stocks were evaluated by limiting dilution on GHOST CXCR4/CCR5 and 293T-CD4-CCR5 cells. In HIV-1 cell capture assays, DC-SIGN or L-SIGN expressing THP-1 transfectants (250,000 cells) were preincubated with pseudotyped HIV-1 (multiplicity of infection ~0.1 with regard to target cell concentration) in a total volume of 0.5 ml for 3 h to allow cellular adsorption of the virus. After 3 h incubation, cells were washed with 2 vol PBS and the THP-1 transfectants were cocultured with Hut/CCR5 targets (100,000 cells) in the presence of 10 μg/ml polybrene in 1 ml cell culture medium. Cell lysates were obtained after 3 d and analyzed for luciferase activity. In contrast, HIV-1 enhancement assays used suboptimal concentrations of virus (typically <0.05 multiplicity of infection) without a wash step. In brief, *DC-SIGN* or *L-SIGN* transfectants (50,000 cells) were incubated with identical virus concentrations (either pseudotyped HIV-1 or replication-competent M-tropic strain HIV-$1_{JR-CSF}$), and after 2 h activated T cells (100,000 cells) were added. Cell lysates were obtained after several days and analyzed for either luciferase activity or p24 antigen levels. T cells were activated by culturing them in the presence of 10 U/ml IL-2 and 10 μg/ml PHA for 2 d.

*Immunohistochemical Analysis.* Staining of the tissue cryosections was performed as described previously (2). 8-μm cryosections of the tissues were fixed in 100% acetone for 10 min, washed with PBS, and incubated with the first antibody (10 μg/ml) for 60 min at 37°C. After washing, the final staining was performed with the ABC-PO/ABC-AP Vectastain kit (Vector Laboratories) according to the manufacturer's protocol. Nuclear staining was performed with hematoxylin.

## Results

*Genomic Map of DC-SIGN and L-SIGN.* A fine map of the *DC-SIGN/L-SIGN* gene locus was determined using information from the human BAC clone CTD-2102F19 sequence, which is now available in GenBank/EMBL/DDBJ (accession no. AC008812; Fig. 1). *DC-SIGN* and *L-SIGN* are positioned in a head-to-head orientation 15.7 kb apart. RH mapping indicated that *DC-SIGN* and *L-SIGN* are located on chromosome 19p13.2-3, near the marker D19S912 (lod score values >11.1) with *DC-SIGN* positioned more telomeric. In agreement with the RH data, the D19S912 marker is found at a distance of ~37 kb centromeric to *L-SIGN* on the BAC sequence.

Soilleux et al. (5) reported a *DC-SIGNR* cDNA clone that contained eight exons with an additional 3′ intron spliced out of the 3′ untranslated region (UTR) compared with the cDNA clone described by Yokoyama-Kobayashi et al. (4). Our RT-PCR experiments on different tissues (liver, pancreas, lung, and placenta) showed that the splice variant described by Soilleux et al. is consistently present but only as a minor transcript, whereas the major transcript consists of seven exons (data not shown). Also, a transcript missing exons 2 and 6, as described by Yokoyama-Kobayashi et al. (4), was extremely rare in our hands.

Northern hybridization data (see below) indicated that *DC-SIGN* mRNA is 3 kb longer than that reported previously (3, 5). We found that this difference is due to the presence of an additional 3-kb UTR in exon 7. Indeed, there was no canonical polyadenylation signal in either of the previously published *DC-SIGN* cDNA sequences, and a search of GenBank/EMBL/DDBJ sequences revealed short polyadenylated ESTs with putative poly(A) signal

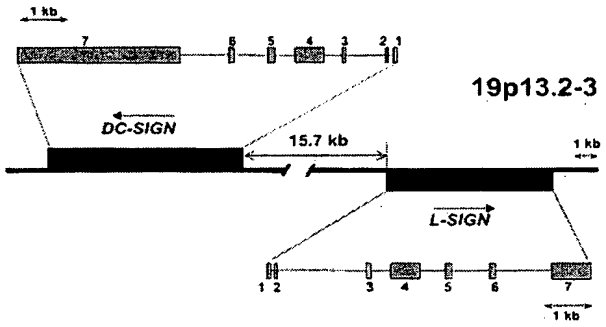


**Figure 1.** Schematic representation of the *DC-SIGN/L-SIGN* genetic map. Physical distances and gene orientation are based on the sequence provided from BAC clone CTD-2102F19 (GenBank/EMBL/DDBJ accession no. AC008812).

motifs mapping 3 kb downstream of the alleged 3′ end of *DC-SIGN*. RT-PCR experiments indicated that those ESTs correspond to *DC-SIGN* mRNA (data not shown). Based on these findings, we conclude that the full *DC-SIGN* transcript contains the additional 3 kb, resulting in a total of 4.3 kb.

*Polymorphism in Exon 4 of L-SIGN.* Exon 4 of both *DC-SIGN* and *L-SIGN* contains repeats of 69 bp that encode repeating units of 23 amino acids. These repeats form a neck between the carbohydrate recognition domain and the transmembrane domain of the SIGN molecules. The *L-SIGN* cDNA clone isolated from placental mRNA contained the entire coding region of the gene, but only six full repeats were present in the sequence corresponding to exon 4, in contrast to seven full repeats identified in the cDNA reported by Soilleux et al. (5). This indicated that the repeat region of *L-SIGN* is polymorphic. Analysis of exon 4 in 350 Caucasian individuals showed the presence of seven alleles based on number of repeats (ranging from three to nine), the most common of which was the allele containing seven repeats (Table I). Strikingly, analysis of *DC-SIGN* exon 4 in 150 Caucasians did not reveal any variability.

*Northern Blot Analysis of DC-SIGN and L-SIGN.* *L-SIGN* mRNA exhibits ~90% similarity to *DC-SIGN* mRNA over the entire coding region, but there is only 53% similarity between exons 2 of the genes. Therefore, exon 2 sequence was used to generate a probe (84 nt) that was *L-SIGN* specific in Northern blot analysis. The probe hybridized to mRNA of ~1.9, 2.6, and 4.2 kb in size in liver and lymph node, and a weak 1.9-kb band was detected in thymus (Fig. 2 A). The 1.9-kb band, which is prominent in lymph node and fetal liver, corresponds to the predicted size of *L-SIGN*. The upper bands (one of which, 2.6 kb, is substantial in adult liver) are likely to be alternative transcripts, but RACE and RT-PCR techniques have not indicated the presence of UTRs varying in length nor alternative splice variants. Therefore, we cannot exclude the possibility that a gene(s) with homology to *L-SIGN* and precisely the same expression pattern is present in humans, but a thorough search for such genes in the sequence databases has been unsuccessful. Finally, a polymorphism in the *L-SIGN* gene (e.g., exon 4 repeat expansion or alteration in the polyadenlation signal motif) could possibly explain the larger transcript size.

Northern blots were reprobed with a 1.2-kb fragment containing the entire coding sequence of *DC-SIGN*, which recognizes both *DC-* and *L-SIGN* mRNA due to their high sequence similarity (Fig. 2 B). Once again, the bands representing *L-SIGN* transcripts were observed in liver, lymph node, and fetal liver. Additionally, a 4.3-kb transcript representing *DC-SIGN* was detected in monocyte-derived DCs and lymph node, and to a lesser extent, in placenta, spleen, thymus, and possibly liver.

*L-SIGN* mRNA was also detected in placenta and DCs using a more sensitive RT-PCR technique, in agreement with previously reported data (5), but the level of expression in these tissues is too low to be detected by Northern hybridization. The probe which recognizes both *DC-SIGN* and *L-SIGN* transcripts with nearly equal sensitivity clearly indicated differential tissue distribution of the two gene products: *L-SIGN* is primarily transcribed in liver and lymph node, whereas *DC-SIGN* is specifically expressed in DCs and tissues that accommodate DCs (Fig. 2; reference 1). Although both *L-SIGN* and *DC-SIGN* mRNAs are found in lymph node, it is likely that they are expressed by different cell types in this tissue. DCs, which are frequent in lymph node, are the source of *DC-SIGN* mRNA in this tissue (2), but *L-SIGN* mRNA is not detected by Northern blot analysis in DCs, peripheral blood lymphocytes, or spleen (Fig. 2). It is possible that *L-SIGN* expression may be inducible in certain leukocytes during specific stages of activation or, perhaps more likely, endothelial cells of the lymph node may constitutively express this receptor. Char-

**Table I.** *Polymorphism of the Repeat Region in L-SIGN Exon 4*

| No. of repeats | Allele frequency (percent) |
|---|---|
| 3 | 1 (0.3) |
| 4 | 25 (3.6) |
| 5 | 202 (28.9) |
| 6 | 86 (12.2) |
| 7 | 377 (53.9) |
| 8 | 2 (0.3) |
| 9 | 7 (1.0) |

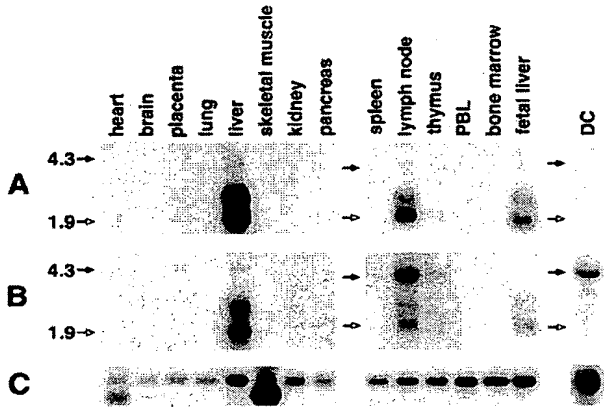


**Figure 2.** Northern blot analysis of *DC-SIGN* and *L-SIGN*. Positions of the 4.3-kb (black arrows) and 1.9-kb (white arrows) sizes are marked on the left. (A) Hybridization with the *L-SIGN*–specific probe indicating expression of the gene in liver, lymph node, and weakly in thymus. (B) Hybridization with the probe recognizing both genes. 4.3-kb bands represent *DC-SIGN* mRNA. The light upper band (~4.2 kb) evident in liver and lymph node using the *L-SIGN*–specific probe (Fig. 3 A) is distinct from *DC-SIGN* mRNA (4.3 kb) due to the specificity of the probe, intensity patterns, and slight differences in size. (C) Reprobing of the blots with the β-actin cDNA control probe.

acterization of the mechanism involved in the differential tissue expression of these two highly homologous molecules will be of particular interest.

*L-SIGN Is Expressed by Human LSECs and Not by DCs.* To identify the cells expressing L-SIGN molecules in vivo, we performed immunohistochemical analysis using a pair of anti–DC-SIGN mAbs, one of which, AZN-D3, cross-reacted with L-SIGN, whereas another, AZN-D1, was DC-SIGN specific (Fig. 3 A). As expected from the Northern blot analysis, poor staining of liver tissue was observed using the DC-SIGN–specific mAb AZN-D1 (Fig. 3 B), and the rare cells detected with this antibody are probably DCs residing in liver. In contrast, the mAb AZN-D3 brightly stained cells lining the sinusoids of the liver (Fig. 3 B). mAbs against the endothelial cell–specific marker CD31 gave a similar staining pattern on serial liver sections (data not shown), suggesting that L-SIGN is expressed by LSECs. To support this idea, primary human LSECs were distinguished from the other hepatic cells by uptake of OVA, which is a unique characteristic of LSECs (15), and were tested for expression of L-SIGN directly. Staining of LSECs with polyclonal anti–L-SIGN antibodies indicated that L-SIGN is expressed exclusively by these cells in liver (Fig. 3 C).

Both AZN-D1 and AZN-D3 stained lymph node equally well (data not shown), but without sufficient definition to determine whether cellular staining patterns differed between the two antibodies. However, using L-SIGN–specific polyclonal antibodies, we found that L-SIGN is not expressed by monocyte-derived DCs (Fig. 3 D), which supports conclusions from the Northern blot analysis. Therefore, it is likely, that DC-SIGN and L-SIGN are expressed by different types of cells in the lymph node.

*L-SIGN Binds ICAM-3 and HIV-1 gp120.* We predicted that L-SIGN and DC-SIGN would bind similar ligands given the nearly identical amino acid sequence of their extracellular domains. Both ICAM-3 and HIV-$1_{MN}$ gp120 have been shown to bind with high affinity to DC-SIGN in a $Ca^{2+}$-dependent manner (1–3). Using a flow cytometry–based adhesion assay (11), K562 cells transfected

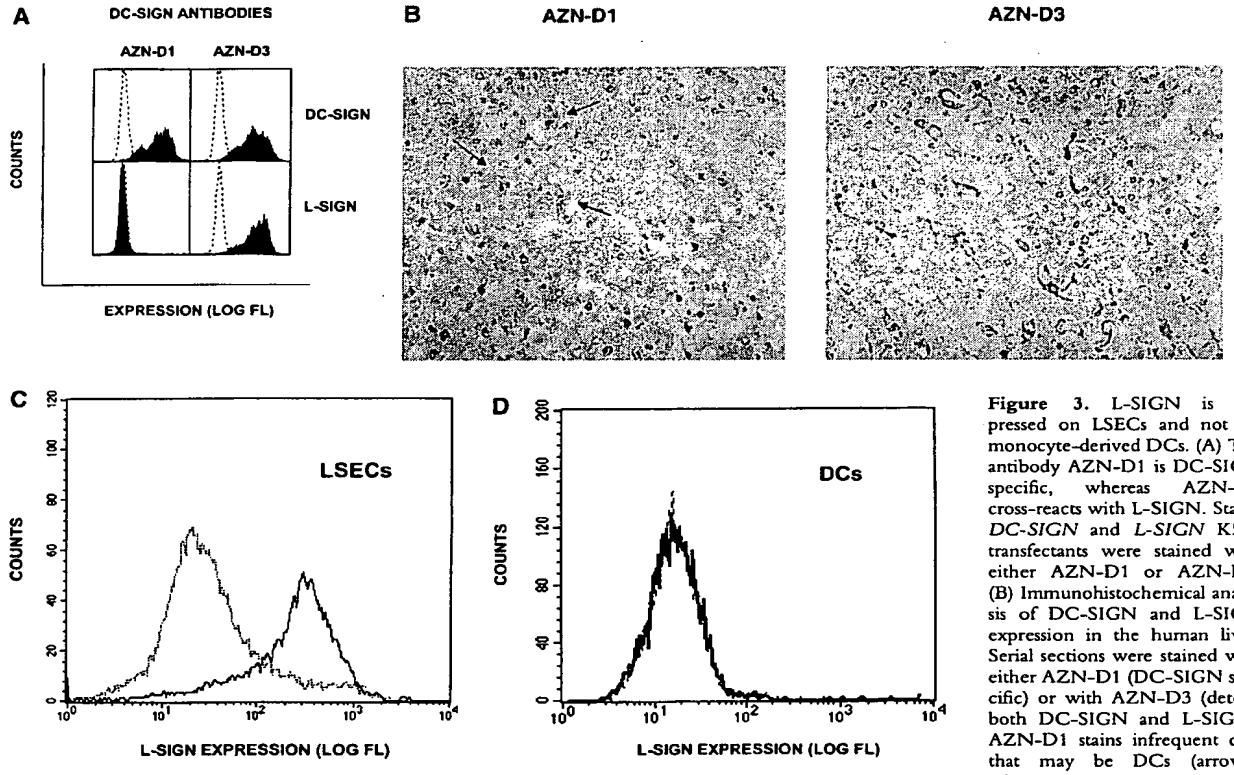


**Figure 3.** L-SIGN is expressed on LSECs and not on monocyte-derived DCs. (A) The antibody AZN-D1 is DC-SIGN specific, whereas AZN-D3 cross-reacts with L-SIGN. Stable *DC-SIGN* and *L-SIGN* K562 transfectants were stained with either AZN-D1 or AZN-D3. (B) Immunohistochemical analysis of DC-SIGN and L-SIGN expression in the human liver. Serial sections were stained with either AZN-D1 (DC-SIGN specific) or with AZN-D3 (detects both DC-SIGN and L-SIGN). AZN-D1 stains infrequent cells that may be DCs (arrows), whereas AZN-D3 stains cells lining sinusoids. (C) Expression of L-SIGN in liver is restricted to LSECs. 1 d after isolation, primary human liver cells were incubated with fluorochrome-labeled OVA. L-SIGN expression was determined by indirect immunofluorescence using an L-SIGN–specific polyclonal antibody. Cells that have taken up OVA (LSECs) and those that did not take up OVA (hepatocytes and other resident hepatic cells) are represented by solid and broken lines, respectively, by gating on the respective cell populations. $2 \times 10^5$ cells were analyzed. (D) L-SIGN is not expressed by monocyte-derived DCs. Immature DCs, cultured from monocytes in the presence of GM-CSF and IL-4, do not stain with anti–L-SIGN polyclonal antibody, as determined by FACScan™ analysis. Solid line indicates staining with anti–L-SIGN polyclonal serum, whereas broken line (hidden under solid lane) represents staining with rabbit preimmune serum.

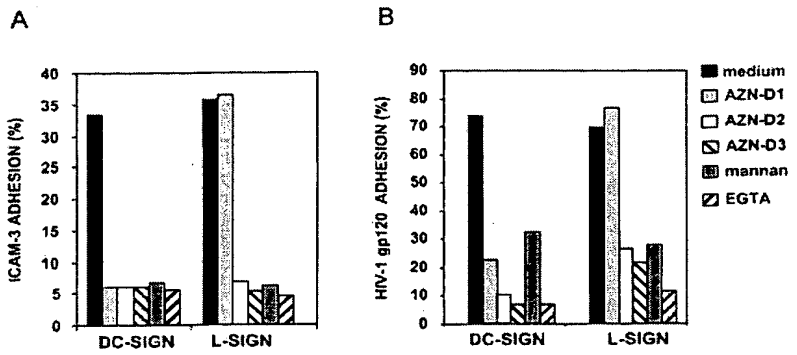


**Figure 4.** L-SIGN binds ICAM-3 (A) and HIV-1 gp120 (B). Adhesion of ICAM-3 and gp120 to the K562–L-SIGN and K562–DC-SIGN cells was measured with the fluorescent bead adhesion assay (reference 11). The y-axis represents the percentage of cells binding ligand-coated fluorescent beads. The L-SIGN cross-reacting mAbs AZN-D2 (20 μg/ml) and AZN-D3 (20 μg/ml) inhibit the adhesion of ICAM-3 and gp120 to L-SIGN, in contrast to the DC-SIGN–specific mAb AZN-D1 (20 μg/ml). Adhesion of both ICAM-3 and gp120 to the K562 transfectants is also inhibited by either 20 μg/ml mannan or 5 mM EGTA. Adhesion of both ligands to mock transfectants was <5%. One representative experiment out of three is shown (SD < 5%).

with *L-SIGN* were shown to bind ICAM-3 with high affinity (Fig. 4 A). The L-SIGN–mediated binding was inhibited by the DC-SIGN/L-SIGN–specific mAbs AZN-D2 and AZN-D3, mannan, or EGTA, but not by the DC-SIGN–specific mAb AZN-D1, demonstrating that L-SIGN functions as a mannose-binding C-type lectin with a high affinity for ICAM-3. As predicted by the high homology to DC-SIGN, L-SIGN was also able to bind to HIV-$1_{MN}$ gp120 in a manner similar to that observed for DC-SIGN (1) (Fig. 4 B). Mock transfected cells did not bind either ICAM-3 or HIV-$1_{MN}$ gp120 (data not shown).

*L-SIGN Enhances HIV-1 Infection.* High affinity binding of L-SIGN to HIV-1 gp120 raised the possibility that, like DC-SIGN, L-SIGN might bind infectious HIV-1 and enhance infection of target cells in trans. To test the role of L-SIGN as a transreceptor in HIV-1 infection, THP-1 cells expressing either DC-SIGN or L-SIGN were pulsed with single round infectious HIV-luciferase pseudotyped with M-tropic HIV-$1_{JRFL}$ envelope glycoprotein, washed to remove unbound virus, and incubated with target cells permissive for HIV-1 infection. Infection was evaluated after 3 d. Both the *L-SIGN*– and *DC-SIGN*–transfected THP-1 cells captured infectious HIV-1 and transmitted the virus to target cells, while mock transfected THP-1 cells did not (Fig. 5 A).

Next we investigated whether L-SIGN would be able to capture a limiting concentration of HIV-1 and efficiently present the virus to the permissive cells promoting infection. HEK293T cells expressing DC-SIGN or L-SIGN, or mock

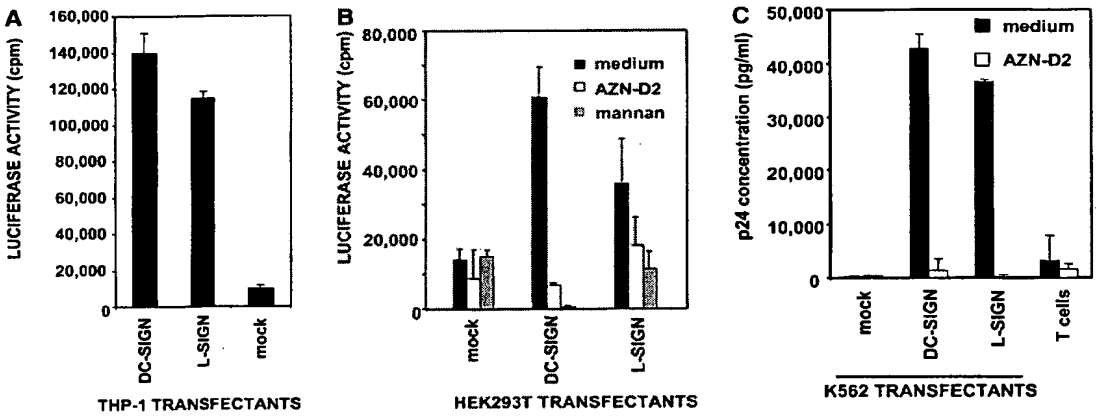


**Figure 5.** L-SIGN captures and enhances infection of T cells with HIV-1 in trans. (A) L-SIGN captures HIV-1 and transmits it to target cells. Stable DC-SIGN– or L-SIGN–expressing THP-1 transfectants were preincubated with HIV-luc/JRFL pseudovirions to allow capture of the virus. Cells were washed and THP-1 transfectants were cocultured with Hut/CCR5 target cells. Cell lysates were obtained after 3 d and analyzed for luciferase activity. For each of the coculture conditions employed, mock infected controls were uniformly <100 cps in activity. Each data set represents the mean of four separate wells of infected cells. One representative experiment out of two is shown. (B) L-SIGN enhances infection of T cells by pseudotyped HIV-1. HEK293T cells were transiently transfected with cDNA encoding DC-SIGN, L-SIGN, or empty vector. Control cells were preincubated with 20 μg/ml AZN-D2 or 20 μg/ml mannan. Low amounts of pseudotyped HIV-$1_{ADA}$ were added together with activated T cells as described previously (reference 1). Infectivity was determined after 2 d by measuring luciferase activity. One representative experiment of two performed is shown. Each experiment was done in triplicate wells. (C) L-SIGN enhances infection of T cells by replication competent HIV-1. Stable K562 transfectants of both L-SIGN and DC-SIGN were incubated with low virus concentrations of replication-competent M-tropic strain HIV-$1_{JR-CSF}$ ($TCID_{50}$ 100/ml). To determine the specificity, cells were preincubated with AZN-D2 (20 μg/ml). After 2 h, activated T cells were added as described previously (reference 2). Culture supernatants were collected at day 14 after K562-T cell coculture and HIV-1 production was measured using ELISA to determine p24 antigen levels. In control experiments, the same amount of virus was added directly to T cells. One representative experiment out of three is shown. Each data set represents the mean of three separate wells of infected cells.

transfected cells were incubated with low titers of HIV-luciferase pseudotyped with HIV-$1_{ADA}$ envelope glycoprotein. The unwashed cells were then cocultured with activated T cells. Minimal infection of target cells was observed from mock transfected HEK293T cells pulsed with HIV-1 (Fig. 5 B). However, HEK293T cells transfected with L-SIGN enhanced HIV-1 infection of T cells in trans, similar to DC-SIGN (Fig. 5 B). The DC-SIGN–mediated enhancement was inhibited with the cross-reactive AZN-D2 antibody, while partial inhibition was observed for L-SIGN, possibly because of some difference in the reactivity of this antibody to the two SIGN molecules that was evident under the conditions employed in this experiment. Mannan efficiently inhibited enhancement by both SIGN molecules.

Similar experiments to evaluate the ability of L-SIGN to enhance HIV-1 infection of T cells were performed using replication-competent virus. K562 cells transfected with *L-SIGN*, *DC-SIGN*, and empty vector were incubated with the M-tropic HIV-$1_{JR\text{-}CSF}$ strain at low virus concentrations for 2 h and subsequently cocultured with activated T cells (Fig. 5 C). No viral replication was observed using mock transfected K562 cells, while *L-SIGN* transfectants transmitted HIV-1 to target cells, resulting in viral replication with nearly the same efficiency as *DC-SIGN* transfectants. Almost complete inhibition of HIV-1 replication with the DC-SIGN/L-SIGN–specific antibody AZN-D2 indicated the specificity of these receptors to enhance HIV-1 infection. Thus, non-DC lineage cells expressing L-SIGN within liver and possibly in lymph node may also have the ability to capture and transmit HIV-1 to lymphocytes.

## Discussion

The homologous human C-type lectins DC-SIGN and L-SIGN appear to be the products of a recent gene duplication. The corresponding proteins share the same domain organization and overlapping, if not completely identical, ligand specificity. The most diverse region of these molecules occurs in their cytoplasmic tails (5). It has been suggested that DC-SIGN–associated HIV-1 may be internalized, protecting it from degradation or inactivation (1). If so, the sequence variation in the cytoplasmic region of L-SIGN relative to DC-SIGN could affect the level of receptor internalization and viral uptake, perhaps explaining the consistent differences in efficiency of HIV-1 infection enhancement observed in our experiments between *DC-SIGN* and *L-SIGN* transfectants (Fig. 5).

Another obvious difference in *SIGN* genes is the repeat polymorphism in exon 4 of *L-SIGN*, which is conserved in *DC-SIGN* (Table I). The neck domain of L-SIGN may contain from three to nine repeats, while DC-SIGN always consists of seven repeats among the Caucasians tested. It is not clear whether the differences in exon 4 diversity of these genes is because of some distinction in the physical feature(s) of the genes or to selective processes acting on the genes differentially. The neck domain may be involved in oligomerization of the receptors (5) and variable numbers of repeats could potentially affect functional characteristics of the L-SIGN molecule, particularly in heterozygotes where heterooligomers might be present. However, our preliminary data indicated no difference between L-SIGN molecules containing six or seven repeats in ligand binding or in HIV-1 capture and enhancement experiments.

Although the *SIGN* genes have maintained sequence and functional similarity over their evolutionary history, regulatory elements determining their tissue distribution have evolved along unique paths. Northern blot analysis of mRNA expression clearly indicated expression of *DC-SIGN* in monocyte-derived DCs and in tissues where DCs reside, whereas expression of *L-SIGN* in DCs was undetectable (Fig. 2). Further, L-SIGN was not detected on monocyte-derived DCs using antibodies specific to L-SIGN (Fig. 3 C). Thus, it is most likely that unique cell types in the lymph node express one but not both SIGN molecules: L-SIGN could be expressed by endothelial cells, as it is in liver, whereas DC-SIGN is expressed by DCs in the T cell area of lymph node (2).

Liver sinusoids are specialized capillary vessels characterized by the presence of resident macrophages adhering to the endothelial lining. The LSEC–leukocyte interactions, which require expression of adhesion molecules on the cell surfaces, appear to constitute a central mechanism of peripheral immune surveillance in the liver (15). The mannose receptor as well as other costimulatory receptors such as MHC class II, CD80, and CD86, are known to be expressed on LSECs and to mediate the clearance of many potentially antigenic proteins from the circulation in a manner similar to DCs in lymphoid organs (15). L-SIGN may fit in this category of receptors on LSECs, as its tissue location and ligand-binding properties strongly implicate a physiologic role for this receptor in antigen clearance, as well as in LSEC–leukocyte adhesion. The high expression of ICAM-3 on apoptotic cells (16) may provide the means by which these cells are trapped by L-SIGN–expressing cells in the liver and subsequently cleared.

The mannose glycans present on gp120 appear to mediate HIV-1 adhesion to the SIGN molecules, although the contribution of the gp120 polypeptide backbone is not excluded (1). Several in vitro studies have shown that highly glycosylated HIV-1 gp120 is a strong ligand for a variety of mannose-binding lectins (17–22). Although the carbohydrate structures on the HIV envelope could be nonspecifically recognized by host lectins, the physiological consequences of such recognition will be specified by the functions of the binding molecule. The SIGN molecules are the first membrane-associated lectins identified to date that enhance HIV-1 infection. Interestingly, the expression of L-SIGN in liver sinusoids suggests that LSECs, which are in continual contact with passing leukocytes, can capture HIV-1 from the blood and promote transinfection of T cells. Moreover, prior studies have indicated that LSECs themselves may be susceptible to HIV-1 infection (23, 24). Thus, it is possible that L-SIGN promotes infection of these cells, thereby establishing a reservoir for production of a new virus to pass on to T lymphocytes trafficking through the liver sinusoid.

Additional functional studies are necessary for understanding the normal physiologic role of L-SIGN and its possible role in HIV-1 pathogenesis. Its ability to enhance transinfection of T cells suggests that L-SIGN may contribute to HIV-1 susceptibility. Alternatively, if a physiologic function of L-SIGN involves antigen clearance, this receptor could play a protective role in clearance of the virus from the circulation. A clearer understanding of this receptor may provide insight into its potential use in novel therapy against HIV-1.

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## References

1. Geijtenbeek, T.B., D.S. Kwon, R. Torensma, S.J. van Vliet, G.C. van Duijnhoven, J. Middel, I.L. Cornelissen, H.S. Nottet, V.N. KewalRamani, D.R. Littman, et al. 2000. DC-SIGN, a dendritic cell-specific HIV-1-binding protein that enhances trans-infection of T cells. *Cell.* 100:587–597.
2. Geijtenbeek, T.B., R. Torensma, S.J. van Vliet, G.C. van Duijnhoven, G.J. Adema, Y. van Kooyk, and C.G. Figdor. 2000. Identification of DC-SIGN, a novel dendritic cell-specific ICAM-3 receptor that supports primary immune responses. *Cell.* 100:575–585.
3. Curtis, B.M., S. Scharnowske, and A.J. Watson. 1992. Sequence and expression of a membrane-associated C-type lectin that exhibits CD4-independent binding of human immunodeficiency virus envelope glycoprotein gp120. *Proc. Natl. Acad. Sci. USA.* 89:8356–8360.
4. Yokoyama-Kobayashi, M., T. Yamaguchi, S. Sekine, and S. Kato. 1999. Selection of cDNAs encoding putative type II membrane proteins on the cell surface from a human full-length cDNA bank. *Gene.* 228:161–167.
5. Soilleux, E.J., R. Barten, and J. Trowsdale. 2000. DC-SIGN, a related gene DC-SIGNR, and CD23 form a cluster on 19p13. *J. Immunol.* 165:2937–2942.
6. Chomczynski, P. 1992. One-hour downward alkaline capillary transfer for blotting of DNA and RNA. *Anal. Biochem.* 201:134–139.
7. Sallusto, F., and A. Lanzavecchia. 1994. Efficient presentation of soluble antigen by cultured human dendritic cells is maintained by granulocyte/macrophage colony-stimulating factor plus interleukin 4 and downregulated by tumor necrosis factor α. *J. Exp. Med.* 179:1109–1118.
8. Romani, N., S. Gruner, D. Brang, E. Kampgen, A. Lenz, B. Trockenbacher, G. Konwalinka, P.O. Fritsch, R.M. Steinman, and G. Schuler. 1994. Proliferating dendritic cell progenitors in human blood. *J. Exp. Med.* 180:83–93.
9. Lub, M., S.J. van Vliet, S.P. Oomen, R.A. Pieters, M. Robinson, C.G. Figdor, and Y. van Kooyk. 1997. Cytoplasmic tails of β1, β2, and β7 integrins differentially regulate LFA-1 function in K562 cells. *Mol. Biol. Cell.* 8:719–728.
10. Cecilia, D., V.N. KewalRamani, J. O'Leary, B. Volsky, P. Nyambi, S. Burda, S. Xu, D.R. Littman, and S. Zolla-Pazner. 1998. Neutralization profiles of primary human immunodeficiency virus type 1 isolates in the context of coreceptor usage. *J. Virol.* 72:6988–6996.
11. Geijtenbeek, T.B., Y. van Kooyk, S.J. van Vliet, M.H. Renes, R.A. Raymakers, and C.G. Figdor. 1999. High frequency of adhesion defects in B-lineage acute lymphoblastic leukemia. *Blood.* 94:754–764.
12. Hegenbarth, S., R. Gerolami, U. Protzer, P.L. Tran, C. Brechot, G. Gerken, and P.A. Knolle. 2000. Liver sinusoidal endothelial cells are not permissive for adenovirus type 5. *Hum. Gene Ther.* 11:481–486.
13. Hild, M., O. Weber, and H. Schaller. 1998. Glucagon treatment interferes with an early step of duck hepatitis B virus infection. *J. Virol.* 72:2600–2606.
14. Connor, R.I., B.K. Chen, S. Choe, and N.R. Landau. 1995. Vpr is required for efficient replication of human immunodeficiency virus type-1 in mononuclear phagocytes. *Virology.* 206:935–944.
15. Knolle, P.A., and G. Gerken. 2000. Local control of the immune response in the liver. *Immunol. Rev.* 174:21–34.
16. Moffatt, O.D., A. Devitt, E.D. Bell, D.L. Simmons, and C.D. Gregory. 1999. Macrophage recognition of ICAM-3 on apoptotic leukocytes. *J. Immunol.* 162:6800–6810.
17. Ezekowitz, R.A., M. Kuhlman, J.E. Groopman, and R.A. Byrn. 1989. A human serum mannose-binding protein inhibits in vitro infection by the human immunodeficiency virus. *J. Exp. Med.* 169:185–196.
18. Gattegno, L., A. Ramdani, T. Jouault, L. Saffar, and J.C. Gluckman. 1992. Lectin-carbohydrate interactions and infectivity of human immunodeficiency virus type 1 (HIV-1). *AIDS Res. Hum. Retroviruses.* 8:27–37.
19. Hammar, L., I. Hirsch, A. Machado, J. De Mareuil, J. Baillon, and J.C. Chermann. 1994. Lectin effects on HIV-1 infectivity. *Ann. NY Acad. Sci.* 724:166–169.
20. Saifuddin, M., M.L. Hart, H. Gewurz, Y. Zhang, and G.T. Spear. 2000. Interaction of mannose-binding lectin with primary isolates of human immunodeficiency virus type 1. *J. Gen. Virol.* 81:949–955.
21. Ushijima, H., H.C. Schroder, S. Poznanovic, M.J. Gasic, E. Matthes, and W.E. Muller. 1992. Inhibition of human immunodeficiency virus-1 infection by human conglutinin-like protein: in vitro studies. *Jpn. J. Cancer Res.* 83:458–464.
22. Milone, M.C., and P. Fitzgerald-Bocarsly. 1998. The mannose receptor mediates induction of IFN-α in peripheral blood dendritic cells by enveloped RNA and DNA viruses. *J. Immunol.* 161:2391–2399.
23. Steffan, A.M., M.E. Lafon, J.L. Gendrault, C. Schweitzer, C. Royer, D. Jaeck, J.P. Arnaud, M.P. Schmitt, A.M. Aubertin, and A. Kirn. 1992. Primary cultures of endothelial cells from the human liver sinusoid are permissive for human immunodeficiency virus type 1. *Proc. Natl. Acad. Sci. USA.* 89:1582–1586.
24. Housset, C., E. Lamas, V. Courgnaud, O. Boucher, P.M. Girard, C. Marche, and C. Brechot. 1993. Presence of HIV-1 in human parenchymal and non-parenchymal liver cells in vivo. *J. Hepatol.* 19:252–258.